**Abstract**

This study presents a comprehensive analysis of morphological variations in the teeth of *Tragelaphus scriptus* and *Tragelaphus pricei*. Utilizing a combination of image processing techniques and advanced statistical analyses, we aimed to quantify and compare the morphological shapes of teeth from these two species.

**Introduction**

Understanding the morphological variation in teeth among mammalian species provides critical insights into their evolutionary history and adaptation strategies. This research focuses on the comparative analysis of *Tragelaphus scriptus* and *Tragelaphus pricei*, aiming to identify significant morphological differences through a novel combination of image processing and statistical analysis. These species, chosen for their evolutionary significance and distinct habitats, offer an opportunity to explore the relationship between morphology and environmental adaptation.

**Methodology**

Data Collection

High-resolution black and white images of the teeth from both species were collected, encompassing six tooth types (LM1, LM2, LM3, UM1, UM2, UM3). The images were collected from museum collections and digitalized for analysis.

Image Processing

Using a combination of image processing techniques, each tooth image was converted into a format suitable for detailed morphological analysis. This process standardized the images across the dataset, facilitating a consistent comparison of shape characteristics.

Shape Extraction and Standardization

The methodological core involved extracting the tooth shape from the images without using landmark-based geometric morphometrics. Instead, shape extraction focused on identifying and isolating the tooth outline using edge detection algorithms. This approach allowed for the capture of the entire tooth contour as a continuous shape, which was then standardized to facilitate direct comparison across samples. Standardization involved scaling the shapes to a uniform size, effectively removing size as a confounding factor and focusing the analysis on shape variation.

Statistical Shape Analysis

Shape analysis was conducted through a sequence of steps designed to quantitatively assess and compare the morphological characteristics of teeth between the two species. This involved the application of shape descriptors and statistical techniques to analyze the extracted shapes. Principal component analysis (PCA) was utilized to reduce dimensionality and to identify the main modes of shape variation within the dataset. The differences in tooth shape between species were then quantitatively assessed using statistical methods that we could use for shape data, including multivariate analysis techniques and permutation tests to evaluate the significance of observed differences.

Statistical Significance Testing

The statistical significance of the observed morphological differences between *Tragelaphus scriptus* and *Tragelaphus pricei* was determined using multivariate and non-parametric methods.

1. Hotelling's T-square Test

Principal component analysis (PCA) scores for each tooth type were compiled into a combined dataset, separating samples into two groups based on their species. Hotelling's T-square test was then applied to these groups, aiming to identify significant multivariate differences in tooth shape. This test offered a comprehensive statistical assessment, taking into account the multidimensional nature of the shape data. The results from Hotelling's T-square test provided initial evidence of the morphological divergence between the two species. To complement the Hotelling's T-square test, permutation testing was conducted. This non-parametric approach involved randomly shuffling the group labels (species assignments) of the combined PCA scores dataset thousands of times (1,000 for Hotelling's T-square significance and 100,000 for distance-based permutation testing), each time recalculating the test statistic under the null hypothesis that there is no difference between species. The permutation tests served two purposes: firstly, to validate the significance of the Hotelling's T-square test results, and secondly, to perform a distance-based permutation test that considered both shape and size differences as delineated by the pairwise distances matrix. The Hotelling's T-square statistic is calculated as:

*Formula*

1. Distance-Based Permutation Testing

The distance-based permutation testing extended the analysis by focusing on the pairwise distances between teeth shapes, encapsulating both size and shape variations. For each tooth type, the average within-species distances and the average between-species distances were calculated. The test statistic, *S*, was derived from these distances to quantify the morphological disparity between species. Permuting the species labels and recalculating *S* for numerous simulations provided a distribution of *S* under the null hypothesis. This approach offered a robust mechanism to assess the likelihood of observing the empirical value of *S* by chance, thereby estimating a p-value for the test.

**Conclusion**

The combined use of Hotelling's T-square test and permutation testing in this study not only underscored the morphological differences between *T. scriptus* and *T. pricei* but also reinforced the statistical robustness of these findings. By employing both parametric and non-parametric methods, the analysis offered a comprehensive and reliable assessment of the shape differences, paving the way for a deeper understanding of their biological and evolutionary significance.